Effects of body condition score at parturition and postpartum supplemental fat on adipose tissue lipogenic activity of lactating beef cows[1]

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ABSTRACT: Three-year-old Angus × Gelbvieh beef cows nutritionally managed to achieve a BCS of 4 ± 0.07 (479.3 ± 36.3 kg of initial BW) or 6 ± 0.07 (579.6 ± 53.1 kg of initial BW) at parturition were used in a 2-yr experiment (n = 36/yr) to determine the effects of BCS at parturition and postpartum lipid supplementation on cow adipose tissue lipogenesis. Beginning 3 d postpartum, cows within each BCS were randomly assigned to be fed hay and a low-fat control supplement or supplements with either cracked high-linoleate safflower seeds or cracked high-oleate safflower seeds until d 60 of lactation. Diets were formulated to be isonitrogenous and isocaloric, and safflower seed diets provided 5% DMI as fat. Adipose tissue biopsies were collected near the tail-head region of cows on d 30 and 60 of lactation. Dietary treatment did not affect ($P \geq 0.43$) adipose tissue lipogenesis. Body condition score at parturition did not affect acetate incorporation into lipid ($P = 0.53$) or activity of acetyl CoA carboxylase ($P = 0.77$) or fatty acid synthase ($P = 0.18$). Lipoprotein lipase activity and palmitate incorporation into triacylglycerol tended to be greater ($P = 0.06$), and palmitate esterification into total acylglycerols was greater ($P = 0.01$) in cows with a BCS of 4 at parturition. Mean activity of acetyl-CoA carboxylase ($P < 0.001$), lipoprotein lipase ($P = 0.01$), and rate of palmitate incorporation into monoacylglycerol ($P = 0.02$), diacylglycerol ($P = 0.001$), triacylglycerol ($P = 0.003$), and total acylglycerols ($P = 0.002$) were greater at d 30 than d 60, suggesting a greater proclivity for fatty acid biosynthesis and esterification by adipose tissue at d 30 of lactation. Although dietary lipid supplementation did not affect adipose tissue lipogenesis, results suggest that cows with a BCS of 4 at parturition have a greater propensity to deliver exogenously derived fatty acids to the adipocyte surface and incorporate preformed fatty acids into acylglycerols as stored adipocyte lipid. Additionally, cows in early lactation seemed to be able to synthesize and incorporate more fatty acids into stored lipid than cows during peak lactation.

Key words: beef cattle, body condition score, lactation, lipid supplementation, lipogenesis

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INTRODUCTION

During early to peak lactation, the nutrient demands of the mammary gland exceed those of the rest of the body, resulting in increased lipid mobilization from adipose tissue and decreased lipid synthesis for body reserves (Barber et al., 1997). A divergence of lipogenic activity toward milk fat synthesis in the mammary gland and away from lipid deposition in adipose tissue occurs, which results in lower BCS of the animal (Smith and Walsh, 1988). The change in physiological state induced by lactation alters adipocyte metabolism to support mammary function (Bauman and Currie, 1980; McNamara et al., 1987). Although the inherent homeorhetic regulation involved with lactation makes repartitioning of nutrients away from the mammary gland difficult, provision of certain dietary fatty acids has been associated with repartitioning of nutrients to support specific productive functions (McNamara et al., 1995; Chilliard, 1993). Bottger et al. (2002) attributed maintenance of greater BCS in lactating beef cows to supplementation of linoleic acid, whereas dietary oleic acid increased milk fat synthesis.

Optimal reproductive performance in beef cows is achieved when cows are at or near a BCS of 5 (Morrison et al., 1999; Hess et al., 2005). Therefore, increasing the condition of thin lactating beef cows by repartitioning

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nutrients toward adipose tissue reserves rather than milk fat synthesis could lead to improved reproduction (Houghton et al., 1990) and decreased maintenance requirements (Wagner et al., 1988).

Our hypothesis was that dietary supplementation of specific fatty acids during early lactation would mediate responses in adipose tissue for synthesis and storage of fatty acids. Our objective was to evaluate the effects of BCS at parturition and supplementation of cracked high-linoleic or cracked high-oleic acid safflower seeds on activity of fatty acid synthase, acetyl-CoA carboxylase, and lipoprotein lipase, as well as acetate incorporation into lipid and palmitate esterification in vitro in adipose tissue of lactating beef cows.

MATERIALS AND METHODS

General

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for the study. Cows were managed as described by Lake et al. (2005). Briefly, in a 2-yr experiment (n = 36/yr), 3-year-old Angus × Gelbvieh beef cows (n = 72) were managed nutritionally to achieve a BCS (1 = emaciated to 9 = obese; Wagner et al., 1988) of 4 ± 0.07 (479.3 ± 36.3 kg of initial BW) or 6 ± 0.07 (579.6 ± 53.1 kg of initial BW) at parturition. Beginning 3 d postpartum, cows were placed into 1 of 6 pens (6 animals per pen) with individual feeding stanchions and fed twice daily.

Diets were hay (2.13% of BW during yr 1 and 2.03% of BW during yr 2) plus a low-fat control supplement (0.57% of BW during yr 1 and 0.30% of BW during yr 2) or supplements with either high-linoleate (hay at 2.32% of BW and supplement at 0.39% of BW during yr 1; hay at 2.03% of BW and supplement at 0.23% of BW during yr 2) or high-oleate cracked safflower seeds (hay at 2.32% of BW and supplement at 0.40% of BW during yr 1; hay at 2.03% of BW and supplement at 0.24% of BW during yr 2) until d 60 of lactation.

Results of previous research at the University of Wyoming indicated that cows of similar genetics produced 9 kg of milk during peak lactation (Bottger et al., 2002). Therefore, diets (Table 1) were formulated to meet the energy requirements of a 544-kg beef cow producing 9 kg of milk at peak lactation. Diets were formulated to provide equal quantities of N and TDN within each year. Dietary ingredients were analyzed for CP (Leco FP-528; Leco Corp., St. Joseph, MO), crude fat (2050 Soxtec Avanti Auto Control Unit; Foss Tecator, Eden Prairie, MN) and fatty acids via direct transesterification (Whitney et al., 1999) with methanolic HCl (Murrieta et al., 2003). Dietary CP was greater in yr 2 due to differences in hay used during yr 1 (bromegrass hay; 8.5 % CP) vs. yr 2 (foxtail millet hay; 10.6 % CP). Dietary TDN was similar between years, and lipid supplemented diets were formulated to be isolipidic, providing 5% DMI as fat.

Sampling

On d 30 and again on d 60, each cow was injected s.c. with approximately 400 mg of lidocain hydrochloride (Vedco, Inc., St. Joseph, MO) as a local anesthetic to desensitize a 10-cm area adjacent to the tail-head region. Adipose tissue biopsies (approximately 5 g) were removed (Rule and Beitz, 1986) and immediately placed in sterile Krebs-Ringer bicarbonate buffer (pH 7.4, 37°C). One-half of the adipose tissue was transported in Krebs-Ringer bicarbonate buffer to the laboratory (within 20 min) for determination of in vitro acetate and palmitate incorporation into total lipid. Remaining adipose tissue was immediately snap-frozen in liquid N and stored at –80°C for later analysis of lipogenic enzyme activity. In vitro lipogenesis and activity of enzymes involved in lipogenesis were determined in order to more fully investigate the potential metabolic response in cows to lipid supplementation and BCS. Preliminary analysis of lipoprotein lipase, fatty acid synthase, and acetyl-CoA carboxylase activity conducted in our laboratory showed no difference (data not shown) between fresh tissue and tissue snap frozen and stored for 80 d.

Lipoprotein Lipase Activity

Lipoprotein lipase was analyzed according to the procedure of Andersen et al. (1996). Total lipoprotein lipase was released from tissues by homogenizing (Tekmar Tissuemizer; Tekmar Tissuemizer Co., Cincinnati, OH) 200 mg of adipose tissue in 2.4 mL of Krebs-Ringer phosphate buffer (pH 7.4) that contained 80 μL of heparin (4.0 mg/mL) and incubated for 30 min at 37°C. Homogenates were centrifuged (1,300 × *g*; Beckman TJ-6, Beckman Instruments, Inc., Fullerton, CA) for 10 min at 4°C, and then 100 μL of aqueous supernatant was mixed with 100 μL of substrate and incubated for 30 min at 37°C. Triolein was used as the substrate, and (9, 10-^{3}H-oleate)-triolein (Perkin Elmer Life Sciences, Boston, MA) was used as the radiotracer. Ovine serum, heated to 60°C for 10 min to inactivate endogenous lipases, was used as the source of apolipoprotein-CII. Reactions were terminated by addition of 3.3 mL of chloroform:methanol:heptane (1.25:1.41:1, vol/vol/vol) and 1 mL of 0.05 *M* bicarbonate to extract fatty acids hydrolyzed by lipoprotein lipase. Samples were vortexed, centrifuged at 1,300 × *g*, and radioactivity was counted in 1.0 mL of the upper phase by liquid scintillation spectroscopy (Beckman LS 1701 Liquid Scintillation System; Beckman Instruments). Activity of lipoprotein lipase was expressed as nanoequivalents of fatty acids released per minute per gram of adipose tissue.

Fatty Acid Synthase Activity

Fatty acid synthase was analyzed according to the procedure of Vernon and Taylor (1986). Snap frozen adipose tissue (1 g) was sliced and immediately placed

Table 1. Ingredient and chemical composition of diets consumed by lactating beef cows[1]

Item	Yr 1 diet			Yr 2 diet		
	Control	High-linoleate	High-oleate	Control	High-linoleate	High-oleate
	Ingredient, % of DM					
Hay[2]	79.3	85.3	85.4	87.2	89.7	89.6
Cracked high-linoleate safflower seeds	—	11.8	—	—	8.1	—
Cracked high-oleate safflower seeds	—	—	9.6	—	—	7.6
Soybean meal	2.8	—	2.1	0.7	—	0.6
Molasses	0.8	0.8	0.8	0.6	0.6	0.6
Beet pulp pellets	15.0	—	—	10.0	—	—
Mineral supplement[3]	2.1	2.1	2.1	1.6	1.6	1.6
	Chemical composition, % of DM					
CP	10.4	10.2	10.4	11.2	11.4	11.4
TDN[4]	70.6	71.1	72.0	69.7	70.1	70.1
Crude fat	1.2	5.0	5.0	2.2	5.0	5.0
	Fatty acid profile of diet, g/100 g of fatty acid					
16:0	28.7	9.9	7.8	19.8	10.0	8.0
18:0	5.6	3.2	0.3	2.7	3.2	0.2
18:1	10.1	10.2	73.2	10.4	10.3	71.3
18:2	20.3	69.7	10.4	22.4	68.1	10.9
18:3	6.6	0.6	0.1	1.7	0.4	0.6

[1]Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544-kg beef cow producing 9 kg of milk during peak lactation. Lipid-supplemented diets were isolipidic and formulated to provide 5% DMI as fat.
[2]Bromegrass hay (CP = 8.5%) was fed in yr 1, foxtail millet hay (CP = 10.8%) was fed in yr 2.
[3]Mineral supplement contained 9% Ca, 15% P, 9% NaCl, 3.7% Mg, 1,265 ppm of Zn, 2,500 ppm of Cu, 5 ppm of Se, 220,500 IU/kg of vitamin A, 110,250 IU/kg of vitamin D, and 110 IU/kg of vitamin E.
[4]TDN for hay samples was estimated from ADF values (Linn and Martin, 1989), whereas tabular values (NRC, 1982) were used to calculate TDN of supplemental ingredients.

in a glass test tube (15 × 85 mm) with 2 mL of homogenization buffer (pH 7.4; 300 m*M* of sucrose, 30 m*M* of Tris-HCl, 1 m*M* of EDTA). Tubes were kept on ice throughout the entire procedure. Tissue samples were completely homogenized (Tekmar Tissuemizer) and centrifuged (1,300 × *g*) for 15 min at 4°C. Aqueous infranatant (150 μL) was filtered through glass wool and mixed with fatty acid synthase assay buffer [850 μL; 0.3 m*M* of NAD phosphate (**NADPH**), 1 m*M* of EDTA, 1 m*M* dithiothreitol, 0.1 m*M* acetyl-CoA, and 0.1 m*M* malonyl-CoA] in a UV cuvette. Change in absorption at 340 nm due to loss of NADPH was determined spectrophotometrically (Beckman DU 640; Beckman Instruments). Disappearance of NADPH in the absence of malonyl and acetyl-CoA was measured, and no oxidation of NADPH was observed. Fatty acid synthase activity was expressed as nanomoles of NADPH lost per minute per gram of adipose tissue.

Acetyl-CoA Carboxylase Activity

Acetyl-CoA carboxylase was analyzed according to the procedure of Vernon and Taylor (1986). Snap frozen adipose tissue (0.5 g) was completely homogenized and centrifuged as described for fatty acid synthase. Portions (150 μL) of each sample were incubated in citrate incubation buffer (350 μL; 20 m*M* of citrate, 20 m*M* of $MgCl_2$, 0.2 m*M* of EDTA, 2 m*M* of reduced glutathione, 2.5 mg/mL of BSA) at 37°C for 30 min. Additional samples were incubated without citrate, and used as the assay control (basal activity). Immediately after citrate buffer incubation, acetyl-CoA reaction buffer (850 μL; 20 m*M* of citrate, 2.5 m*M* of ATP, 12.5 m*M* of $NaHCO_3$, 0.2 m*M* of acetyl-CoA, 1.25 μCi of $NaH^{14}CO_3$/mL (Perkin Elmer Life Sciences) was added and incubated for 90 s at 37°C. The reaction was terminated after 90 s by addition of 200 μL of 6 *N* HCl. Vials were heated at 80°C for 1 h with lids removed to allow nonincorporated radio-labeled bicarbonate to dissipate. Samples were vortexed, centrifuged at 1,300 × *g*, and radioactivity counted in 1.0 mL of the upper phase by liquid scintillation spectroscopy. Acetyl-CoA carboxylase activity was expressed as nanomoles of radiolabeled bicarbonate incorporated per minute per gram of adipose tissue.

Acetate and Palmitate Incorporation into Lipid

For in vitro assays of acetate incorporation into total lipids (Rule et al., 1987) and palmitate (Bouyekhf et al., 1992) esterification, 100 mg of minced fresh adipose tissue was incubated for 90 min in 3.0 mL of Krebs-Ringer bicarbonate buffer that contained 10 m*M* of glucose and 1,000 μU/mL of insulin. In addition, 0.75 m*M* of potassium palmitate and 1.0 μCi of ^{14}C-palmitate or 5.0 m*M* of acetate and 0.5 μCi of ^{14}C-acetate (Perkin Elmer Life Sciences) were added to palmitate and acetate buffers, respectively. Incubations were conducted in 16 × 125 mm siliconized screw-cap tubes in an orbital shaker waterbath (120 rpm; Laboratory-line, Newark, DE) at 37°C. Reactions were terminated by first rinsing tissue slices with a mixture of Krebs-Ringer bicarbonate buffer and 2% BSA to rid tissue slices of excess

isotope, and then total lipids were extracted overnight with chloroform:methanol:water (1:2:0.8,vol/vol/vol). Radioactivity in the total lipid fraction was determined by liquid scintillation spectroscopy. Acetate and palmitate incorporation rates were expressed as nanomoles of acetate or palmitate converted to total lipid per minute per gram of lipid. To exclude the presence of nonmetabolically active tissues, rates were expressed as grams of lipid extracted from tissue used in the assay. For the palmitate assay, half of the $CHCl_3$ was removed and placed into a separate vial and stored at –4°C for fractionation into mono, di-, and triacylglycerol by TLC. Palmitate (200 μL) in chloroform was dried, reconstituted in 20 μL of $CHCl_3$, and blotted onto channeled TLC plates (Silica-Gel G Channeled plates, Analtech, Newark, DE). Petroleum ether:diethyl ether:glacial acetic acid (85:15:1, vol:vol:vol) was used as the developing solvent to separate neutral and polar lipid fractions. Lipid bands were identified with iodine vapors, and individual fractions were scraped into scintillation vials for determination of radioactivity.

Statistical Analyses

All data were analyzed as a split-plot with a 2 × 3 arrangement of treatments in a randomized complete block design using the MIXED procedures of SAS (SAS Institute, Inc., Cary, NC). Year was used as a block, and the model included the effects of BCS at parturition, dietary treatment, day of sampling, and all possible interactions. The effects of BCS at parturition and dietary treatment were tested using individual cow as the RANDOM statement. Cow within BCS at parturition × dietary treatment was used as the SUBJECT and day of sampling as the REPEATED statement. Using likelihood ratio testing, an AR-1 structure was deemed most appropriate for the within-subjects effects (the effects associated with day of sampling). Comparisons of main effects and interactions were determined using least square means. During the first year of the study, one calf died; however, the cow was mechanically milked twice daily to enable her to stay on the experiment. Observations from this cow were tested for normality to ensure mechanical milking did not affect milk production. During the second year of the study, one cow-calf pair was removed due to death of the calf. Necropsies performed at the Wyoming State Veterinary Laboratory revealed that the deaths of the calves were not attributed to the study; consequently, least squares means were reported. Best linear unbiased predictor estimates from the random statement were tested for normal distribution, and all enzymatic and esterification data required log transformation. Statistical probabilities in the tables were derived from log transformed data; however, data presented in the tables were nontransformed. Pearson correlations also were calculated to evaluate relationships between variables for lipogenic activity and production traits.

Table 2. Main effects of body condition score at parturition on adipose tissue lipogenesis in lactating beef cows[1]

	BCS			
Item	4	6	SEM[2]	*P*-value[3]
Acetate incorporation into lipid[4]	1.99	1.15	0.63	0.53
Activity of:				
Acetyl-CoA carboxylase[5]	11.30	12.22	2.27	0.77
Fatty acid synthase[6]	1.29	0.83	0.24	0.18
Lipoprotein lipase[7]	29.95	24.87	2.31	0.06
Palmitate esterification[8] into:				
Monoacylglycerol	7.45	3.60	0.94	0.01
Diacylglycerol	16.82	13.67	2.12	0.30
Triacylglycerol	39.11	25.71	4.95	0.06
Total	63.87	43.29	7.13	0.01

[1]Cows were nutritionally managed to achieve a BCS of 4 ± 0.07 or 6 ± 0.07 at parturition (Lake et al., 2005). Refer to Table 1 for description of diets fed during early lactation.

[2]Greatest SEM was reported (BCS 4 at parturition, n = 36; BCS 6 at parturition, n = 35).

[3]Statistical probabilities were determined from log transformed data. Mean values reported are from nontransformed data.

[4]Acetate incorporation into lipid was expressed as nmol of acetate converted to total lipid·min^{-1}·g $lipid^{-1}$.

[5]Acetyl-CoA carboxylase activity was expressed as nmol of HCO_3- converted to malonyl-CoA·min^{-1}·g adipose $tissue^{-1}$.

[6]Fatty acid synthase activity was expressed as nmol of NAD phosphate disappearance·min^{-1}·g adipose $tissue^{-1}$.

[7]Lipoprotein lipase activity was expressed as nEq fatty acid hydrolyzed·min^{-1}·g adipose $tissue^{-1}$.

[8]Palmitate esterification into monoacylglycerol, diacylglycerol, and triacylglycerol was expressed as nmol of palmitate incorporated into acylglycerols·min^{-1}·g adipose $tissue^{-1}$.

RESULTS AND DISCUSSION

Effect of BCS at Parturition on Adipose Tissue Lipogenic Activity

Enzyme activity data were expressed on a per gram of tissue basis to determine responses within the mass of adipose tissue, which permitted evaluation on a whole animal basis. Mean incorporation of acetate into total lipid ($P = 0.53$) and activity of acetyl-CoA carboxylase ($P = 0.77$) and fatty acid synthase ($P = 0.18$) were not affected by BCS (Table 2). However, lipoprotein lipase activity ($P = 0.06$) tended to be greater in BCS 4 cows compared with BCS 6 cows. Likewise, rates of palmitate incorporation into monoacylglycerols ($P = 0.01$) and total acylglycerols ($P = 0.01$) were greater, and incorporation into triacylglycerol tended to be greater ($P = 0.06$) for BCS 4 cows compared with BCS 6 cows. A BCS at parturition × day of sampling interaction was noted ($P = 0.05$) for palmitate incorporation into monoacylglycerol because cows with BCS of 4 at parturition had greater ($P < 0.001$) rates of palmitate incorporation into monoacylglycerol at d 30 than d 60 (11.02 vs. 3.88 nmol·min^{-1}·g $lipid^{-1}$), whereas cows in a BCS of 6 at parturition had similar ($P = 0.67$) rates of palmitate incorporation into monoacylglycerol at d 30 compared with d 60 (4.01 vs. 3.19 nmol·min^{-1}·g $lipid^{-1}$).

Lipoprotein lipase catalyzes hydrolysis of fatty acids from circulating lipoprotein triaclyglycerols. The free

fatty acids would then be available for transport into the adipocyte and incorporation into triacylglycerol for storage. Therefore, greater lipoprotein lipase activity in BCS 4 cows would infer that if availability of circulating triacylglycerols to adipocytes was increased, there would be a greater supply of FFA presented to the adipocyte surface. Furthermore, increased palmitate esterification into acylglycerols would indicate greater proclivity for BCS 4 cows to increase adipose tissue storage, supporting maintenance of condition in BCS 4 cows during the course of the current study, whereas cows with BCS 6 lost condition (Lake et al., 2005). No difference was detected in milk yield or energy between BCS 4 or 6 cows in the current study (Lake et al., 2005), suggesting BCS 4 cows utilized nutrients more efficiently. Similarly, Houghton et al. (1990) suggested that cows maintained in suboptimal condition lose less BW in the form of palpable adipose tissue and therefore would be more efficient in nutrient use than cows maintained in above optimal condition.

In nonruminant animals, lipoprotein lipase is tightly regulated by circulating levels of insulin (Braun and Severson, 1992; Mead et al., 2002). Faulconnier et al. (1994) concluded that, although less sensitive to insulin, lipoprotein lipase from bovine adipose tissue is regulated by insulin similarly to humans and rats. Likewise, Andersen et al. (1996) reported lipoprotein lipase activity in adipose tissue of growing lambs reflected energy balance. McFadden et al. (1990) suggested that energy balance might influence the number of insulin receptors in adipose tissue of growing lambs. Elevated levels of circulating insulin might increase lipoprotein lipase activity. In the current study, circulating insulin concentrations were not affected ($P = 0.27$; data not shown) by cow BCS; however, BCS 4 cows may have had greater sensitivity to insulin or an increase in insulin receptors, resulting in increased lipoprotein lipase activity and a potentially increased efficiency of tissue accretion. Thus, greater lipoprotein lipase activity in cows with a BCS of 4 at parturition would infer that if rate of circulating chylomicrons increased at the adipocyte, there would be a greater supply of FFA presented to the adipocyte surface. Moreover, if a greater proportion of dietary triacylglycerols could be diverted to adipose tissue during lactation, cows in low BCS could increase in body condition more rapidly.

The glycerol-3 phosphate pathway is the predominant means by which di- and triacylglycerols are synthesized in bovine adipocytes (Vernon, 1981). Through this pathway phosphatidic acid is produced, and then the phosphate group is hydrolyzed yielding diacylglycerol, which is then esterified to a third fatty acid to produce triacylglycerol. Intracellular adipose tissue lipolysis would result in hydrolysis of fatty acids from either di- or triacylglycerols resulting in production of FFA, monoacylglycerols, and glycerol as an intermediate. Although monoacylglycerols and diacylglycerols may be products of lipolysis, esterification of C^{14} palmitate must occur and precede lipolysis in order to observe monoacylglycerols. Therefore, differences in the rate of incorporation of palmitate into acylglycerol was interpreted to suggest a difference in the overall rate of fatty acid esterification. The greater rate of palmitate esterification into total acylglycerols in vitro would indicate that adipocytes of cows with a BCS of 4 at parturition could incorporate and store more fatty acids in the cell if greater dietary fatty acids were presented to the cell surface. Furthermore, plasma NEFA and β-hydroxybutyrate concentrations tended ($P = 0.08$) to be lower for BCS 4 cows than for BCS 6 cows (Lake et al., 2004), indicating that lipolysis rates may have been less for BCS 4 cows. Hence, greater lipoprotein lipase activity and increased palmitate esterification into acylglycerols for BCS 4 cows is understandable because cows in suboptimal BCS would have a greater need to increase body adipose tissue stores (Wagner et al., 1988).

Effect of Dietary Treatment on Adipose Tissue Lipogenic Activity

A sharp decline in lipogenic activity in adipose tissue during early to peak lactation and a rebound to greater rates during midlactation led McNamara et al. (1995) to suggest that a specific pathway regulates adipose tissue lipogenic activity during lipogenesis. The addition of fat to the diet during early lactation is commonly thought to improve energy balance, resulting in reduced body tissue mobilization (Komaragiri et al., 1998). However, rates of acetate incorporation into fatty acids, activity of fatty acid synthase, and activity of lipoprotein lipase were reported to be functionally nonexistent and unaffected by duodenal rapeseed oil infusion in dairy cows during early lactation (Chilliard et al., 1991). Nonetheless, previous research from our laboratory (Bottger et al., 2002) attributed changes in milk energy and BCS in beef cows to nutrient partitioning facilitated by dietary linoleic acid.

Although Scholljegerdes et al. (2004) reported that intestinal flow of fatty acids was vastly different among cows fed safflower seed supplements similar to those used in the present experiment, our dietary treatments did not affect the activity of lipoprotein lipase ($P = 0.54$), fatty acid synthase ($P = 0.47$), acetyl-CoA carboxylase ($P = 0.80$), acetate incorporation into total lipids in vitro ($P = 0.44$), or palmitate esterification into monoacylglycerols ($P = 0.75$), diacylglycerols ($P = 0.43$), triacylglycerols ($P = 0.64$), or acylglycerols ($P = 0.92$; Table 3). Results from the current study are in agreement with McNamara et al. (1995) who reported no difference in rates of fatty acid esterification at d 60 of lactation due to lipid supplementation.

The endocrine system likely superseded dietary influences on nutrient partitioning to support specific metabolic activities (Bauman and Currie, 1980; Komaragiri et al., 1998). The lack of dietary treatment effect on adipose tissue lipogenic activity from beef cows in the current study was unexpected because previous research by our laboratory (Bottger et al., 2002) suggested

Table 3. Main effects of dietary treatment on adipose tissue lipogenesis in lactating beef cows

	Diet[1]				
Item	Control	High-linoleate	High-oleate	SEM[2]	*P*-value[3]
Acetate incorporation into lipid[4]	2.62	0.79	1.30	0.77	0.44
Activity of:					
Acetyl-CoA carboxylase[5]	9.72	11.32	14.24	2.78	0.80
Fatty acid synthase[6]	1.29	1.10	0.79	0.29	0.47
Lipoprotein lipase[7]	26.48	28.50	27.25	2.74	0.54
Palmitate esterification[8] into:					
Monoacylglycerol	6.15	4.81	5.62	1.15	0.75
Diacylglycerol	16.06	12.53	17.14	2.60	0.43
Triacylglycerol	31.41	33.11	32.71	6.09	0.64
Total	54.05	50.82	55.88	8.76	0.92

[1]Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544-kg beef cow producing 9 kg of milk during peak lactation. Lipid-supplemented diets were formulated to provide 5% DMI as fat. Refer to Table 1 for diet description.

[2]Greatest SEM was reported (control, n = 24; high-linoleate, n = 23; and high-oleate, n = 24).

[3]Statistical probabilities were determined from log transformed data. Mean values reported are from nontransformed data.

[4]Acetate incorporation into lipid was expressed as nmol of acetate converted to total lipid·min^{-1}·g lipid^{-1}.

[5]Acetyl-CoA carboxylase activity was expressed as nmol of HCO_3- converted to malonyl-CoA·min^{-1}·g adipose tissue^{-1}.

[6]Fatty acid synthase activity was expressed as nmol of NAD phosphate disappearance·min^{-1}·g adipose tissue^{-1}.

[7]Lipoprotein lipase activity was expressed as nEq fatty acid hydrolyzed·min^{-1}·g adipose tissue^{-1}.

[8]Palmitate esterification into monoacylglycerols, diacylglycerol, and triacylglycerol was expressed as nmol of palmitate incorporated into acylglycerols·min^{-1}·g adipose tissue^{-1}.

that partitioning of nutrients was influenced by type of fatty acid supplementation. Apparent repartitioning effects were detected at d 90 of lactation in the study of Bottger et al. (2002). Peak milk production in beef cows occurs at about 60 d (NRC, 2000). Perhaps the nutrient demands associated with early lactation masked potential partitioning effects associated with lipid supplementation during the first 60 d of lactation. Alternatively, lack of differences in adipose tissue lipogenic activity would be expected because all diets provided equal N and energy. Nonetheless, the lack of dietary treatment effects on lipogenic enzyme activity and rates of lipid synthesis in vitro were consistent with the lack of change in BCS across dietary treatment (Lake et al., 2005).

Effect of Day of Sampling on Adipose Tissue Lipogenic Activity

In dairy cattle, as peak lactation approaches, activity of lipogenic enzymes in adipose tissue continually decreases (Barber et al., 1997). The NRC (2000) suggests that average peak milk production occurs at d 60 of lactation for beef cows; therefore, decreases in lipogenic activity at d 60 of lactation would be expected. Homeorhetic adaptations coordinate the metabolism of adipose tissue to help meet the energetic demands of lactation, resulting in decreased adipose tissue lipogenesis and increased lipolysis (McNamara and Hillers, 1986a).

In the current study, the rates of acetate incorporation into total lipid ($P = 0.19$) and fatty acid synthase activity ($P = 0.17$) in adipose tissue were not different between days of sampling (Table 4). Activities of acetyl-CoA carboxylase ($P < 0.001$), lipoprotein lipase ($P = 0.01$), and palmitate esterification into acylglycerols ($P < 0.001$ to 0.02) were greater at d 30 of lactation than at d 60 of lactation, suggesting that adipose tissue of cows maintained a greater propensity for fatty acid biosynthesis and substrate esterification at d 30 of lactation. Also, the in vitro assay for palmitate esterification requires palmitate to be transported into the adipocyte, followed by activation by CoA thioesterification before entering the glycerolipid biosynthesis pathway.

The apparent downregulation of the above processes in the cow's subcutaneous adipose tissue indicates that lactation imposes significant demands on metabolism to support milk production in the beef cow. This agrees with the performance data from the current study (Lake et al., 2005) where cows lost more BW from d 30 to 60 of lactation than from parturition to d 30. Furthermore, decreased adipose tissue acetyl-CoA carboxylase activity and esterification of fatty acids in beef cows as peak lactation approached was consistent with results of Barber et al. (1997) with dairy cows. Likewise, McNamara and Hillers (1986b) concluded that rates of lipogenesis and esterification in bovine adipose tissue decrease from the onset of lactation through peak lactation and begin to increase once energy nadir is reached.

Correlations

The decrease in lipoprotein lipase from d 30 to 60 was correlated ($P = 0.03$; $r = -0.26$) with the loss in BW occurring from d 30 to 60. Acetyl-CoA carboxylase

Table 4. Main effects of day of sampling on adipose tissue lipogenesis in lactating beef cows[1]

Item	Day			
	30	60	S[2]	*P*-value[3]
Acetate incorporation into lipid[4]	1.05	2.10	0.57	0.19
Activity of:				
Acetyl-CoA carboxylase[5]	16.10	7.42	1.98	<0.001
Fatty acid synthase[6]	0.82	1.30	0.24	0.17
Lipoprotein lipase[7]	33.72	21.10	2.89	0.01
Palmitate esterification[8] into:				
Monoacylglycerol	7.51	3.54	0.94	0.02
Diacylglycerol	20.79	9.69	2.12	<0.001
Triacylglycerol	41.12	23.70	4.95	0.003
Total	69.92	37.25	7.13	0.002

[1]Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544-kg beef cow producing 9 kg of milk during peak lactation. Refer to Table 1 for diet description.
[2]n = 71.
[3]Statistical probabilities were determined from log transformed data. Mean values reported are from nontransformed data.
[4]Acetate incorporation into lipid was expressed as nmol of acetate converted to total lipid·min^{-1}·g lipid^{-1}.
[5]Acetyl-CoA carboxylase activity was expressed as nmol of HCO_3- converted to malonyl-CoA·min^{-1}·g adipose tissue^{-1}.
[6]Fatty acid synthase activity was expressed as nmol of NAD phosphate disappearance·min^{-1}·g adipose tissue^{-1}.
[7]Lipoprotein lipase activity was expressed as nEq fatty acid hydrolyzed·min^{-1}·g adipose tissue^{-1}.
[8]Palmitate esterification into monoacylglycerol, diacylglycerol, and triacylglycerol was expressed as nmol of palmitate incorporated into acylglycerols·min^{-1}·g adipose tissue^{-1}.

activity was correlated at both d 30 (P = 0.02; r = –0.27) and 60 (P = 0.02; r = –0.28) with loss in BW on the corresponding day. Additionally, palmitate esterification into total acylglycerols on d 30 was correlated (P = 0.02; r = –0.28) with BW loss on d 30. Because acetyl-CoA carboxylase catalyzes the rate-limiting step in fatty acid synthesis, the negative correlation that resulted from decreased enzyme activity along with decreased BW was not surprising. Likewise, the negative correlation that resulted from the decreased rate of palmitate esterification in vitro and BW loss was expected considering fatty acid esterification is an essential step in the glycerolipid biosynthesis pathway to increase adipocyte energy stores of acylglycerols.

In conclusion, our results indicate that specific fatty acids in dietary lipid supplements may not effectively repartition nutrients to adipose tissue during early to peak lactation. The demands of lactation seem to dictate the partitioning of nutrients away from adipose tissue in beef cows. At the onset of lactation, the biochemistry of adipose tissue seems to be altered in support of partitioning nutrients to mammary tissue for synthesis of milk at the expense of body condition. Increased activity of key regulatory lipogenic enzymes for cows in a BCS of 4 at parturition caused enhanced nutrient supply sufficient enough to maintain adipose tissue reserves.

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